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13. ABSTRACT ***(Maximum 200 Words)***

Contact among cells in epithelia plays an important role in regulating cell growth and migration. Cell-cell contact generally suppresses cell division and limits migration, while disruption of cell-cell interactions stimulates cell cycle entry and permits migration. A multiprotein complex relays information about cell-cell contact at the plasma membrane into the nucleus, affecting the expression of genes important for cell division. This protein complex includes the catenins and the tumor suppressor APC. Mutations that dysregulate catenins or that inactive APC occur commonly in epithelial malignancies. We recently discovered that the protein Siah-1 interacts with the APC/catenin complex, and regulates the ubiquitin-dependent turnover of β-catenin through a novel previously unidentified mechanism. We also showed that this pathway is linked to genotoxic injury (DNA damage) responses involving p53, an important tumor suppressor. To understand the in vivo relevance of Siah-1 interactions with the APC/catenin complex, we proposed generating transgenic mice that express genes which modulate this pathway in the prostate gland, thus providing new insights to prostate cancer pathogenesis and metastasis.

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FOREWORD

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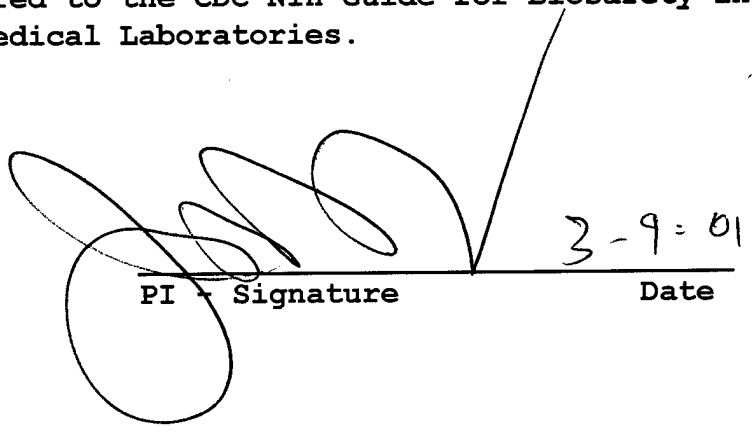

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Final Report
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John C. Reed, MD, Ph.D.

Functions of Beta- and Gamma-Catenins in Prostate Cancer

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INTRODUCTION

The Wnt signaling pathway is conserved from humans to insects and controls a variety of aspects of cell differentiation, migration, and division (reviewed in 1). In humans, multiprotein complexes that include the tumor suppressor APC and the catenin-family proteins constitute essential components of the Wnt pathway. Catenins link the actin cytoskeleton to cell adhesion proteins, including E-cadherin and desmosomal proteins. Loss of these connections, can release β-catenin, allowing it to translocate to the nucleus, bind Tcf/LEF family transcription factors and induce the expression of several genes involved in cell division and oncogenesis, including *C-MYC* and *CYCLIN-D1* (2-4). APC controls the levels of β-catenin, collaborating with the kinase GSK3 and the F-box protein β-Tcrp to induce polyubiquitination and proteosome-dependent degradation of β-catenin. Thus, APC-assisted degradation of β-catenin prevents transactivation of genes that drive the cell cycle. Disruptions of the human equivalent of the Wnt signaling pathway occur commonly in human epithelial malignancies, including loss of E-cadherin expression, mutations that inactivate APC, and mutations that dysregulate catenin-family proteins (reviewed in 5). Defects in the cadherin/catenin/APC axis are often associated with cancer progression to metastatic disease (6, 7).

We have discovered that the protein Siah-1 binds to APC. Siah-1 is an E3 protein, which binds ubiquitin-conjugating enzymes (E2s), targeting them to various substrates in cells and thereby inducing their polyubiquitination and subsequent turnover by the proteosome (8). In contrast to the previously described mechanism for β-catenin degradation which depends on phosphorylation of β-catenin and phosphorylation-dependent interactions of β-catenin with the F-box protein β-Tcrp (9), Siah-1 can promote phosphorylation-independent, β-Tcrp-independent degradation of β-catenin (10). Thus, Siah-1 represents an alternative pathway for controlling signaling through the Wnt-pathway in human cells, and may therefore function as a tumor suppressor that collaborates with APC. Moreover, because Siah-1 expression is induced by p53, this pathway for controlling β-catenin levels is functionally linked to DNA damage responses (9).

To test this hypothesis, we have generated genetically modified mice which lack SIP, a Siah-1 binding protein which links Siah-1 to downstream components of the β-catenin degradation machinery. By studying the physiology of the prostate glands of these mice and by mating them to oncogene-harboring mice, which express SV40 Large T antigen in the prostate, we will gain critical insights into the in vivo significance of the Siah-1 pathway for β-catenin degradation and tumor suppression.

BODY

OBJECTIVES

The revised and approved objectives of our project were to:

1. Generate transgenic mice that overexpress Siah-1 or Siah-1(ΔRING) in the prostate under a probasin-promoter.

2. Examine the histoarchitecture, expression of β-catenin, rates of cell proliferation, and rates of apoptosis in the prostate glands of these mice under physiological conditions and after androgen deprivation.

3. Breed Siah-1 and Siah-1(ΔRING) transgenic mice with SV40-LgT/prostate mice, thus examining whether Siah modulate prostate carcinogenesis and/or metastasis in vivo.

PROGRESS

Objective #1. Generate transgenic mice that overexpress Siah-1 or Siah-1(ΔRING) in the prostate under a probasin-promoter.

We constructed vectors in which cDNAs encoding Siah-1 or Siah-1(ΔRING) are under the control of the probasin promoter. These were transferred into prostate cancer lines to verify that they express properly, before considering microinjection into mouse eggs and generation of the transgenic lines.

We were unable to derive any stably transfected clones of cells from these experiments, indicating that deregulated expression of Siah-1 and Siah-1(ΔRING) is incompatible with cell division or cell survival. Thus, we were forced to consider an alternative approach.

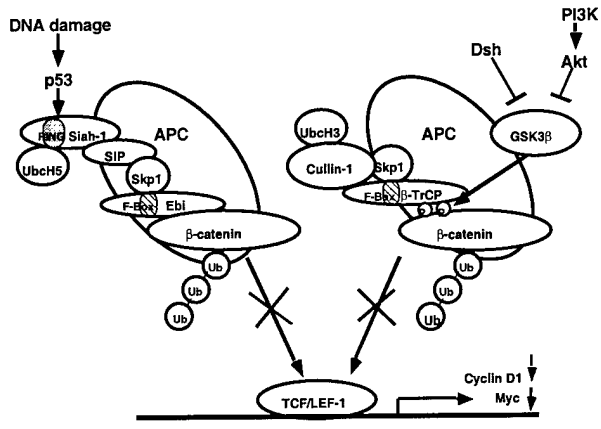

Figure 1. Model of two pathways of β-catenin regulation. Two alternative pathways for regulation of β-catenin levels are presented, involving different F-box proteins (Ebi versus β-TrCP) and possibly different E3 ubiquitin ligases (SSF vs SCF). One pathway (*left*) is initiated by increases in the expression of Siah-family proteins, which can be induced for example by p53 in response to DNA damage, and involves sequential protein interactions with SIP, Skp1, and Ebi. Ebi binds β-catenin, thus recruiting it to the Siah-1/SIP/Skp1 complex for polyubiquitination and subsequent proteosome-mediated

degradation. Siah-1 binds the E2, UbcH5. The other pathway (*right*) is regulated by Wnt-signals (Dsh) and possibly PI3K/Akt. This pathway is phosphorylation-dependent and involves GSK3β-induced phosphorylation of Ser33 and Ser 37 on β-catenin, allowing β-TrCP binding, resulting in recruitment of β-catenin to Skp1/Cullin-1/β-TrCP complexes (SCF). Cullin-1, in collaboration with other proteins not shown, supplies this SCF complex with E2s such as UbcH3. Destruction of β-catenin reduces Tcf/LEF-mediated gene expression, resulting in inhibition of cell proliferation or changes cell fate determination. APC is required for both pathways as a scaffold protein, binding β-catenin via one domain and also binding Siah-1 (left) and GSK3β (right)

We therefore studied how Siah-1 induces degradation of β-catenin. We found that Siah-1 binds to a protein, which we have named SIP for Siah Interacting Protein (10) (*preprint is provided in Appendix*). The SIP protein functions as a molecular bridge, linking Siah to Skp, which in turn bind the F-box protein Ebi, which then binds β-catenin (10). We reasoned therefore that by ablating SIP expression, we could cut-off Siah-1 from downstream steps in the β-catenin degradation pathway, while not perturbing the normal Wnt-signalling pathway that also controls β-catenin degradation (Figure 1).

A targeted insertion of the SIP gene was found during a search of the Lexigen, Inc. database, permitting us to order ES cells which contain a targeted insertion of this gene and thus begin our attempts to generate a SIP knockout mouse. Homozygous SIP(-/-) knock-out mice were recently produced and confirmed by Southern and Northern blotting experiments (performed at Lexigen) to have targeted disruptions of the SIP gene and to lack expression of SIP mRNA. These mice will be critical for future work as we try to understand the significance of SIP for regulation of Siah-1-mediated degradation of β-catenin in vivo.

Objective #2. Examine the histoarchitecture, β-catenin levels, rates of cell proliferation, and rates of apoptosis in the prostate glands of these mice under physiological conditions and after castration.

Not applicable until the animals are transported to our Institute and a mouse colony is established.

Objective #3. Breed Siah-1 and Siah-1(ΔRING) transgenic mice with SV40-LgT/prostate mice, thus examining whether Siah modulates prostate carcinogenesis and/or metastasis in vivo.

A colony of probasin-LgT transgenic mice has been established. These will be mated with SIP (-/-) knock-out mice in the future.

Other Studies. Since it was known that Siah-1 expression can be induced by p53 (11, 12), we were interested to explore whether p53 regulates β-catenin degradation through the Siah-1, SIP, Skp, Ebi pathway outlined in Figure 1. This is an important issue, because: (a) p53 is a tumor suppressor gene which becomes

inactivated in about half of all human cancers, including aggressive prostate cancers; (b) p53 plays an important role in DNA damage responses, linking DNA damage to pathways for cell cycle arrest, apoptosis, and DNA repair; and (c) knowledge about DNA damage response pathways (and defects in them) is critical for understanding why cancers (including prostate cancers) develop resistance to chemotherapy and radiation therapy.

Our studies took several forms (10), which are described in detail in the preprint provided in the Appendix. We found that p53 does indeed induce β-catenin degradation and we presented evidence that the causative mechanism requires Siah, SIP, Skp, and Ebi. Thus, we identified a new pathway that p53 regulates, involving β-catenin degradation. Moreover, we showed that disrupting this pathway causes malignant cells to fail to undergo the cell cycle arrest that p53 normally induces in response to DNA damage. Thus, the pathway is functionally important for stopping cell division after the genome is damaged. Consequently, tumor cells with defects in this pathway might be more genetically unstable, particularly following treatment with DNA-damaging agents.

KEY RESEARCH ACCOMPLISHEMENTS

1. A new pathway linking p53 (DNA damage responses) to β-catenin degradation and cell cycle regulation was discovered and the molecular steps delineated.

2. Knock-out mice were generated lacking the gene encoding SIP (Siah Interacting Protein). These animals will be used for future studies designed to explore the significance of the Siah-pathway for prostate cancer pathogenesis and responses to radiation and chemotherapy.

REPORTABLE OUTCOMES

1. Publications:

The studies in which we describe the original observations linking Siah to a p53-dependent pathway for β-catenin degradation have been submitted for publication. A copy is provided in the appendix section.

> Matsuzawa S, Reed J.C.: Siah-1, SIP, and Ebi Collaborate in a Novel Pathway for β-Catenin Degradation Linked to p53 Responses. Submitted, 2001

2. SIP knock-out mice

3. Cloned cDNAs for wild-type and mutant Siah-1, Siah-2, SIP, Skp1, Ebi, β-catenin.

4. Antibodies to SIP protein.

KEY PERSONNEL (Paid with Grant)

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Helene Baribault
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Marie Josee Bonneau

CONCLUSION

β-catenin serves as a critical relayer of signals from cell-cell adhesion events at the submembranous cytoskeleton to the nucleus, where it controls the expression of genes involved in cell cycle. The factors that control the levels of β-catenin in epithelial cells are complex. We have discovered novel proteins involved in β-catenin degradation. Gaining an improved understanding of the mechanisms relevant to the control of β-catenin in vivo is essential for devising strategies for restoring normal growth control to epithelial malignancies. The work ongoing in this proposal will establish the in vivo significance of novel β-catenin regulating proteins in the prostate of mice.

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